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Case of Retroperitoneal Dedifferentiated Mixed-Type Liposarcoma: Comparison of Proliferative Activity in Specimens from Four Operations

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In a case of retroperitoneal dedifferentiated mixed-type liposarcoma, a dedifferentiated component was observed in the so-called mixed-type liposarcoma consisting of well-differentiated and myxoid components. The proliferative activity was compared among the different components of the tumor by immunohistochemical study using the proliferating cell nuclear antigen (PCNA) and MIB-1 monoclonal antibodies. The dedifferentiated component showed higher positivity than the well-differentiated and myxoid components, and tumor progression was most advanced in the dedifferentiated component. In the chronological examination of each component, the labeling indices of PCNA and MIB-1 were significantly higher at the third recurrence than in the primary lesion in all types, indicating that the proliferative activity of the tumor cells increased gradually. Considering the surgical treatment of liposarcoma, an extended resection encompassing normal adjacent tissues is required in cases containing the dedifferentiated component in comparison to the cases containing only well-differentiated or myxoid components.

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KEY WORDS: retroperitoneal liposarcoma; dedifferentiated liposarcoma; proliferative activity; monoclonal antibody MIB-1; monoclonal antibody PCNA

INTRODUCTION

Liposarcoma is currently classified into the types of well-differentiated, myxoid, round cell, pleomorphic, and dedifferentiated liposarcoma, but very occasional cases (5%–10%) show a combination of two or three components (so-called mixed-type liposarcoma)[1]. When the dedifferentiated component is demonstrated in any of those types, its effects on prognosis or clinical outcome are hard to define. The present case had three types of components (well-differentiated, myxoid, and dedifferentiated) and was diagnosed as dedifferentiated mixed-type liposarcoma. We retrospectively investigated the proliferative activity in specimens, resected at four chronological points, by immunohistochemistry using monoclonal antibodies to proliferating cell nuclear antigen (PCNA) and MIB-1 and examined the effects of repeated recurrences on the malignant progression of the liposarcoma.

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Fig. 1. CT scans of the abdomen at four chronological points: **(a)** the primary tumor in the anterior aspect of the left kidney, **(b)** the first recurrent tumor caudal to the hiatus of the esophagus (arrow), **(c)** the second recurrent tumors posterior to the head of the pancreas (arrow) and near the tail of the pancreas (arrowhead), and **(d)** the third recurrent tumor at the site of left nephrectomy.

CASE REPORT

Surgery

A 52-year-old man with persistent low-grade fever and general fatigue was referred to the Department of Urology of our hospital in December 1994. Laboratory findings showed mild inflammation with a leukocyte count of 11,300/mm^3, C-reactive protein of 2.6 mg/dl, and elevated erythrocyte sedimentation rate (85/125 mm). The serum tumor markers were within normal limits. Computed tomography (CT) of the abdomen revealed a low-density, relatively homogenous, 7.5 × 6.0 cm retroperitoneal tumor in the anterior aspect of the left kidney. On enhanced CT, the tumor was shown to be heterogenous (Fig. 1a). The first operation was performed on January 12, 1995. At laparotomy, a large retroperitoneal mass adherent to the left kidney, which was supplied by the lumbar vessels, extended from above the kidney to the spleen. The mass was totally excised with left nephrectomy. Macroscopically, the resected edge was bimorphic of gray-white and pale yellow. Pathologically it was mixed-type liposarcoma consisting of trimorphic components—the well-differentiated component (Fig. 2a), myxoid component (Fig. 2b), and dedifferentiated component—like malignant fibrous histiocytoma characterized by a storiform growth pattern (Fig. 2c). Therefore, dedifferentiated mixed-type liposarcoma was its final pathological diagnosis. There were some micrometastatic foci in the left kidney.

Sixteen months later, follow-up CT of the abdomen revealed a low-density 3.5 cm mass, caudal to the hiatus of the esophagus (Fig. 1b). At operation in July 1996, the recurrent tumor at the primary site was excised. The tumor measured 4.0 × 3.5 × 1.5 cm; macroscopically, the resected edge was bimorphic of pale yellow and gelatinous. At this first recurrence, the tumor histologically consisted of well-differentiated and myxoid components only, and no dedifferentiated component was found.

Twenty-one months later, follow-up CT of the abdomen demonstrated two masses: one posterior to the head of the pancreas and the other near the tail of the pancreas in the retroperitoneum (Fig. 1c). At this third operation in May 1998, the two separate tumor masses were resected.

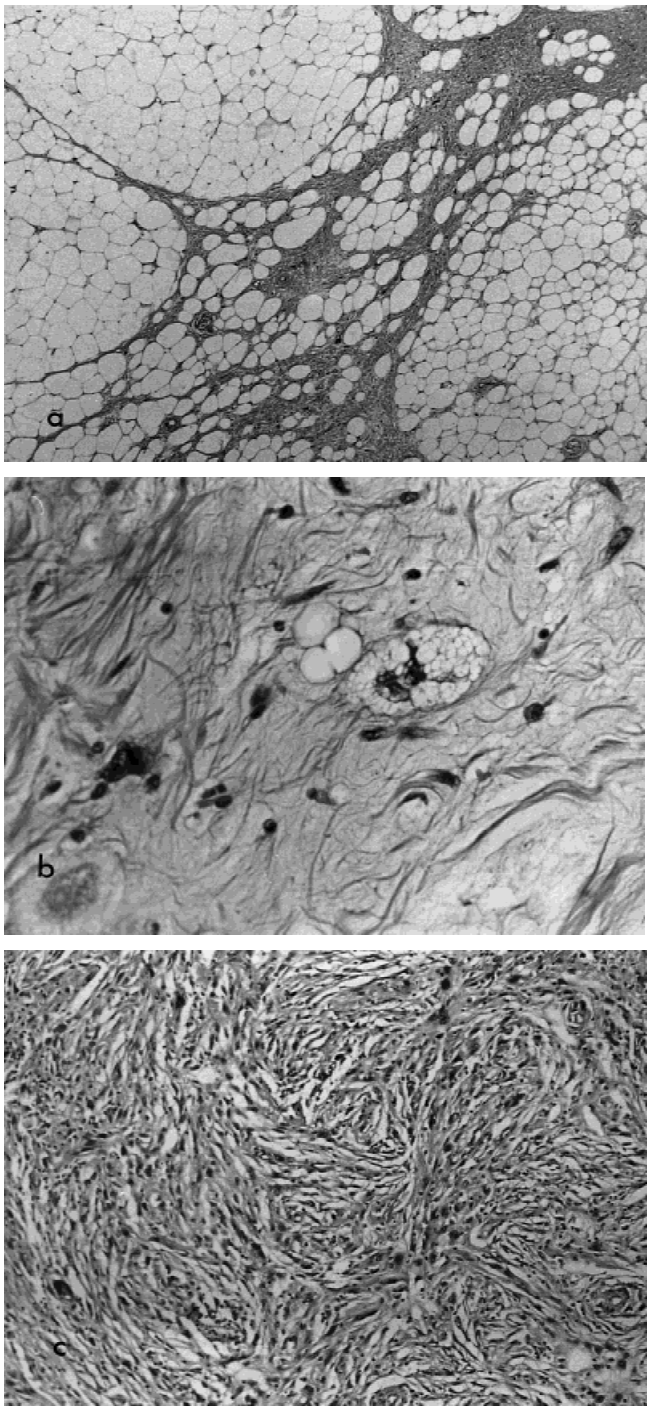

Fig. 2. Microscopic findings: features of **(a)** well-differentiated lipoma-like liposarcoma component (H&E, ×10), **(b)** prominent plexiform capillary pattern in the cellular myxoid liposarcoma component with a lipoblast (H&E, ×100), and **(c)** nonlipogenic dedifferentiated component with features of storiform malignant fibrous histiocytoma (H&E, ×25).

They were 6.0 × 5.0 × 3.0 cm and 4.5 × 3.0 × 2.5 cm, and their components were the same as those at the first recurrence. In this operation, intraoperative procedure for tumor dissection was relatively easy, and macroscopically the dissected margin seemed tumor-free. However, infiltration of tumor cells was histologically observed in the encapsulated area of the tumor margin.

Four months later, a progressing tumor was found on CT at the site of a nephrectomized left kidney (Fig. 1d). At the fourth operation, on September 16, 1998, we found recurrent liposarcoma with direct invasion to the mesentery of the jejunum, the transverse and descending colon, the tail of the pancreas, the left diaphragm, and the left psoas major muscle. Total resection of the mass with fibrotic tissue was performed, with splenectomy, distal pancreatectomy, partial resection of the jejunum, left hemicolectomy, and partial resection of the diaphragm and the left psoas major muscle. Pathological examination revealed tumor cells invading the pancreas, the intestinal wall of the colon and jejunum, the diaphragm, and the psoas major muscle. At the third recurrence, the dedifferentiated component, which had been absent at the first and the second recurrences, was observed again, along with the well-differentiated and myxoid components. The patient's postoperative course has been uneventful for 4 years since the first operation. He has been doing well during the 4 months after discharge. Tumor locations in this care are shown in Figure 3.

MIB-1 and PCNA Immunostaining

Proliferative activity of the tumor cells in all the resected specimens was assessed by immunostaining with the monoclonal antibodies to PCNA (Dako, Carpinteria, CA) and MIB-1 (Immunotech, Marseille Cedex, France). PCNA is originally defined as an intranuclear polypeptide, which synthesizes at its maximum during the S-phase of the cell cycle [2,3], and MIB-1 recognizes the Ki-67 antigen, which is expressed exclusively in the nuclei of proliferating cells (i.e., cells in the G1, S1, G2, and M phases) [4]. All the specimens were fixed in 10% formalin, embedded in paraffin, and cut into 5 μm sections, which were dewaxed, washed with 10 m*M* buffered saline (PBS), and heated in 5 m*M* Tris-HCl buffer (pH 7.6) and 10 m*M* citrate buffer (pH 6.0) in a microwave processor for 15 minutes (3 minutes, 5 times at 750 W). Then the sections were treated with 0.3% hydrogen peroxide in methanol to block endogenous peroxidase activity. After a wash with PBS, normal sheep serum was added to block nonspecific reactions. A 100-fold dilution of the monoclonal antibodies to PCNA and MIB-1 in PBS containing 1% serum albumin was applied to the sections, and they were incubated for 20 minutes at room temperature. After a wash with PBS, immunostaining was done by the avidin-biotin complex technique using biotin-labeled anti–mouse IgG and the peroxidase-labeled streptavidin biotin method.

PCNA- and MIB-1-positive tumor cells were counted in 10 microscopic fields (corresponding to a total of at least 1,000 tumor cells) under high-power magnification

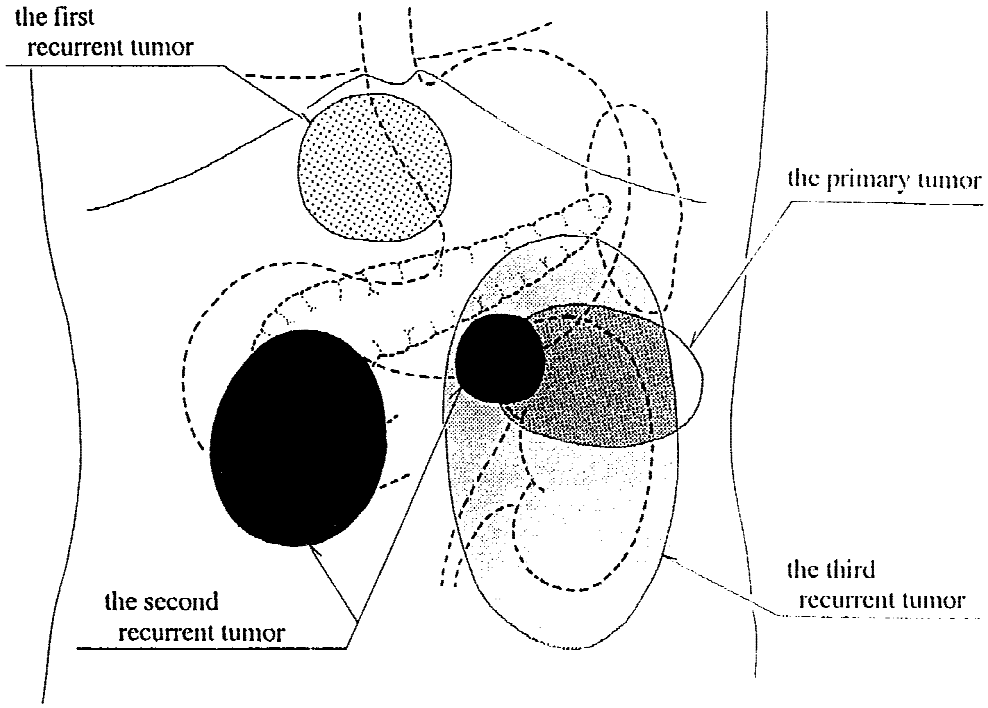

Fig. 3. Schematic drawing of the tumor locations.

(×40 objective lens and ×10 ocular lens). The proportion of PCNA- and MIB-1-positive cells in each component was expressed as the mean number of immunoreactive cells per 100 tumor cells. Data are expressed as the mean ± standard deviation and termed the labeling index. The labeling indices were higher in the dedifferentiated component than in other components (Table I). The indices tended to increase with time in all the components.

DISCUSSION

Histopathological classification of liposarcoma has been diverse because of its complex histological components [5,6]. Enzinger and Weiss [1] classified it into five basic histological categories, namely, well-differentiated, myxoid, round cell, pleomorphic, and the addition of dedifferentiated, which had previously been subclassified under "well-differentiated," with a footnote that very occasional cases (5%–10%) show a combination of two or three components (so-called mixed-type liposarcoma). In 1997, however, Mentzel and Fletcher [7] demonstrated from their clinicopathological and cytogenetic studies that dedifferentiation may occur in myxoid and/or round cell liposarcomas and suggested the close relationships between well-differentiated liposarcoma and myxoid and/or round cell liposarcoma.

The present case was diagnosed as dedifferentiated mixed-type liposarcoma because the dedifferentiated component was observed in the mixed-type liposarcoma consisting of the well-differentiated and myxoid components. Pathologically, there was a transitional zone between the well-differentiated component and the myxoid component; further, the two components were observed in all the specimens resected at four chronological points. These findings support the results of Mentzel and Fletcher [7]. Meis [8] explained that dedifferentiation is a final common pathway for some sarcomas, that is, the tumor cell actually reverses the differentiation process to become a primitive cell and dedifferentiation may occur in any type of liposarcoma.

In the present case, we compared the degree of malignancy among the different types of components by immunohistochemical study and found evidence that the dedifferentiated component showed higher proliferative activity than the well-differentiated and myxoid components and that tumor progression was most advanced in the dedifferentiated component. In the chronological examination of each component, the labeling indices of PCNA and MIB-1 were significantly higher at the third recurrence than in the primary lesion in all the types, indicating that the proliferative activity of the tumor cells increased gradually. Furthermore, the dedifferentiated component, which had been absent at the first and the second recurrences, was again observed at the third recurrence, showing a higher labeling index of PCNA and MIB-1 than the well-differentiated and myxoid components. These findings demonstrated that the degree of malignancy of the mixed-type liposarcoma was enhanced by the repeated recurrences in the present case and fur-

TABLE I. Comparison of Proliferative Activity of the Specimens resected at 4 Chronological Points

	Primary lesion	First recurrence	Second recurrence	Third recurrence
PCNA labeling indices				
Well-differentiated component	8.0 ± 3.7	11.7 ± 3.0	19.6 ± 4.6	21.5 ± 17.8
Myxoid component	23.6 ± 5.2	24.3 ± 5.3	43.8 ± 11.1	50.4 ± 6.6
Dedifferentiated component	34.9 ± 4.7	Not detected	Not detected	71.5 ± 16.3
MIB-1 labeling indices				
Well-differentiated component	1.5 ± 1.0	2.3 ± 0.9	4.1 ± 1.6	3.1 ± 1.5
Myxoid component	2.9 ± 1.5	3.6 ± 1.7	8.6 ± 2.4	12.0 ± 2.5
Dedifferentiated component	12.0 ± 2.6	Not detected	Not detected	20.8 ± 4.1

ther indicated that tumor malignancy is more evident in the liposarcoma with the dedifferentiated component than in those without it. Therefore, in the surgical treatment of liposarcoma containing the dedifferentiated component, an extended resection encompassing normal adjacent tissues is required, compared to the cases of only well-differentiated or myxoid type. Further cell-biological studies of liposarcoma cases are expected, which will enable us to define the significance of dedifferentiation in liposarcoma.

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COMMENTARY

Kato et al. in the above article document a progressive case of retroperitoneal dedifferentiated liposarcoma over a few years. In the third recurrence the dedifferentiated component was proportionately larger and the proliferative indices in all cell types were higher than in the original tumor. The third recurrence best fits anatomically the category of local recurrence. The second recurrence at the esophageal hiatus and a portion of the second recurrence, behind the head of the pancreas, were too far from the primary site to be conveniently classified as local recurrences. Some of these recurrences may, in fact, represent new independent primary tumors of the same histology within a "diseased field" concerning in this case the retroperitoneal adipose tissue. This hypothesis would be in accord with the observed findings, i.e., lack of dedifferentiation in the first and second recurrences (which were probably new primary liposarcomas in the retroperitoneum), and advanced dedifferentiation in the third clinical recurrence, which may have been the first local recurrence from the original tumor.

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